UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
THE ADMINISTRATOR
EPA SCIENCE ADVISORY BOARD

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Note to the Reader:

The attached draft report is a draft report of the EPA Science Advisory Board (SAB). The draft is still undergoing final internal SAB review, however, in its present form, it represents the consensus position of the panel involved in the review. Once approved as final, the report will be transmitted to the EPA Administrator and will become available to the interested public as a final report.

This draft has been released for general information to members of the interested public and to EPA staff. This is consistent with the SAB policy of releasing draft materials only when the Committee involved is comfortable that the document is sufficiently complete to provide useful information to the reader. The reader should remember that this is an unapproved working draft and that the document should not be used to represent official EPA or SAB views or advice. Draft documents at this stage of the process often undergo significant revisions before the final version is approved and published.

The SAB is not soliciting comments on the advice contained herein. However, as a courtesy to the EPA Program Office, which is the subject of the SAB review, we have asked them to respond to the issues listed below. Consistent with SAB policy on this matter, the SAB is not obligated to address any responses that it receives.

1. Has the Committee adequately responded to the questions posed in the Charge?
2. Are any statements or responses made in the draft unclear?
3. Are there any technical errors?

For further information or to respond to the questions above, please contact:

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A DRAFT REPORT OF THE SAB

SUPPLEMENTAL GUIDANCE FOR ASSESSING CANCER SUSCEPTIBILITY FROM EARLY-LIFE EXPOSURE TO CARCINOGENS (SGACS) REVIEW PANEL

EXECUTIVE SUMMARY

The Agency requested that the Science Advisory Board (SAB) conduct this review in an expedited manner and utilize the expertise of two other EPA advisory committees, the FIFRA Scientific Advisory Panel (SAP) and the Children's Health Protection Advisory Committee (CHPAC). By including members of these three EPA advisory bodies in the review of this guidance, the requesting office hoped to benefit from their unique expertise in children's risk assessment and to obtain timely advice.

EPA's Guidelines for Cancer Risk Assessment have undergone several revisions and have been reviewed, fully or in part, by the Science Advisory Board on a number of occasions. This current draft document entitled "Supplemental Guidance for Assessing Cancer Susceptibility from Early-Life Exposure to Carcinogens" (SGACS) represents an attempt by the Agency to be responsive to the recommendations of previous SAB panels. The September 2000 report of the EPA Scientific Advisory Board reviewed the draft revised cancer risk assessment guidelines pertaining to children (http://www.epa.gov/sab/pdf/ec0016.pdf). In that report (p. 34), the SAB suggested, "Quantitatively analyzing the available experimental and epidemiological literature on age dependence in carcinogenesis, in a comprehensive and systematic review, would be very helpful." The SAB review suggested the possibility of incorporating age-dependent susceptibility through age-specific adjustment factors for potency or response to exposures. The research summary presented in the draft Supplemental Guidance and summarized in Tables 1-10 and Figures 1 and 2 responds to this suggestion.

Summary Of EPA's Draft Supplemental Guidance

According to EPA's draft Supplemental Guidance, the approach for assessing cancer risk from early-life exposure to carcinogens includes exposure that is measured or modeled for all lifestages including those during childhood and during adulthood if the data from early-life studies are available and appropriate.

The agency concludes that analysis of the available data supports higher cancer risks from exposures that occur early in life compared to the same exposures during adulthood. Consequently, in the absence of early-life studies on a specific agent under consideration, the Agency generally should use linear extrapolation to lower doses since mutagens, based on mode-of-action data, can give rise to cancers with an apparently low-dose-linear response. Risk estimates that pertain to childhood exposure should be adjusted since risk estimates based on a lifetime-average daily dose do not consider the potential for higher cancer risks from early-life exposure. The following adjustments represent a practical approach that reflects the results of the analysis presented in the Supplemental Guidance, which concluded that cancer risks generally were higher from early-life exposure than from similar exposure durations later in life:

- For exposures before 2 years of age, a 10-fold adjustment.
- For exposures between 2 and 15 years of age, a 3-fold adjustment.
- For exposures after 15 years of age, no adjustment.

The draft Supplemental Guidance concludes that, with regard to modes of action other than mutagenicity, there is insufficient information currently available to determine a general adjustment; consequently, no general adjustment was recommended at this time even though the available science indicates that higher cancer risks sometimes result from early-life exposure. The Agency expects that as other modes of action become better understood, this information will include data on quantitative differences between children and adults, and these differences will be reflected in risk estimates for childhood exposure. The Agency expects to expand the Supplemental Guidance to include other modes of action as they are understood and used in risk assessments.

When the mode of action cannot be established, the current practice of using linear extrapolation to lower doses such that risk estimates are based on a lifetime-average daily dose without further adjustment should be continued and no general adjustment is recommended at this time by the Agency. The result would be expected to produce risk estimates that generally are protective, based on the use of linear extrapolation as a default in the absence of information on the likely shape of the dose-response curve.

SAB RECOMMENDATIONS

The Panel appreciates the Agency's responsiveness to earlier SAB recommendations that the supplemental guidance for assessing cancer susceptibility from early-life exposure to carcinogens be a stand-alone document. Because many parts of the Cancer Guidelines provide the background for the Supplemental Guidance, issuance of the Supplemental Guidance before the Guidelines could be confusing. The Panel, therefore, encourages the Agency to rapidly finalize the Guidelines, and the Supplement soon after, if not concurrently. The Panel wishes to commend the Agency for the hard work reflected in the Supplemental Guidance and is supportive of the approach taken**.** After reviewing the draft Supplemental Guidance and considering public comments, a summary of the panel's response to the charge questions is presented in the following comments and recommendations:

The Panel concurs with the overall approach adopted by the Agency of using adjustment factors to account for increased susceptibility due to early-life exposure, and the values chosen for the cancer slope adjustment factors in the Supplemental Guidance appear to be reasonable from consideration of the literature. However, the Panel suggests that the Agency improve the statistical analysis of the data (as discussed below) and that they provide a more extensive discussion of how the Agency arrived at the choice of the 10X and 3X adjustment factors. The Agency should also make clear that these default adjustment factors would be used only when no data are available to directly assess cancer susceptibility from early-life exposure to a particular

carcinogen. The Agency should consider conducting additional research to address this issue directly as suggested by several public presenters**.**

In this current review activity, the Agency sought the Science Advisory Board's evaluation of the soundness of the Agency's position that the Agency's analysis and the underlying scientific information support the conclusion that there is greater susceptibility for the development of tumors as a result of exposures in early lifestages as compared with adult exposures to chemicals acting through a mutagenic mode of action. The SGACS review panel was specifically asked to respond to the following charge questions that are divided into two parts, (1) questions concerning the supplemental guidance for assessing cancer susceptibility from early-life exposure to carcinogens and (2) other questions:

Summary Response To Specific Charge Questions:

<u>Questions Concerning The Supplemental Guidance For Assessing Cancer Susceptibility From Early-Life Exposure To Carcinogens</u>

1. Please comment on whether the Agency's analysis as applied to chemicals acting through a mutagenic mode of action is accurate, reliable, unbiased and reproducible. Likewise, please comment on whether the underlying scientific information used to develop the guidance is accurate, reliable, unbiased and reproducible. Are there any key studies that the Agency has overlooked in reaching this conclusion?

- The Panel agrees with the Agency that the available science supports the conclusion that early life exposure to carcinogens that act through a mutagenic mode of action increases

susceptibility to carcinogens compared to adult exposures. The Panel notes that a broader look at the scientific literature beyond the studies included in the Supplemental Guidance analysis further strengthen that conclusion.

2. For chemicals acting through non-mutagenic modes of action, the Agency concludes that a range of approaches needs to be developed over time for addressing cancer risks from childhood exposures. Please comment on the Agency's conclusion that the scientific knowledge and data are insufficient at this time to develop generic guidance on how to address these chemicals and that a case-by-case approach is more suitable. Is the SAB aware of any additional data for chemicals acting through non-mutagenic modes of action relevant to possible early lifestage sensitivity?

- The Panel notes that for certain groups of non-mutagenic chemicals (e.g., estrogen receptor agonist/antagonist) there is enough evidence supporting increased susceptibility to cancer with early life exposure that the Agency should include a discussion of these agents in the draft Supplemental Guidance. These chemicals serve as important examples in support of applying a default factor to non-mutagenic carcinogens when the mode of action is unknown.

- Non-mutagenic carcinogens with known mode of action should be assessed on a case-by-case basis as suggested by the Agency.

3. Assuming that it is appropriate to conclude that there is differential lifestage susceptibility to chemicals acting through a mutagenic mode of action, the Agency's guidance uses a default approach that adjusts cancer slope factors (typically from conventional animal bioassays and/or epidemiologic studies of adult exposure) to address the impact of early lifestage exposure. Please comment on whether the approach is justified by the available data? Can the SAB suggest other approaches that might be equal or more appropriate?

- The Panel supports the use of slope factor adjustments in developing default approaches. Application of an adjustment to the adult cancer slope factor seems to be the most transparent and practical approach for risk assessment.

4. When considering differential susceptibility, the Agency's guidance separates the potential susceptible period into two age groups, 0 - 2 years and 2 - 15 years. These groupings were based on biological considerations rather than exposure considerations. The first grouping, 0 - 2 years of age, is meant to encompass a period of rapid development and the second grouping, 2 - 15 years of age, was selected to extend through middle adolescence approximately following the period of rapid developmental changes during puberty. Please comment on the scientific rationale that was used to justify these age groupings. Can the SAB suggest other plausible ways to make these groupings?

- The Panel discussed the Agency age groupings used in the adjustment factor development and reviewed age-specific human vulnerabilities and concluded that it would be useful to include an additional age grouping (age 9 –15) to recognize the potentially important

vulnerabilities during puberty. Thus four age groupings would be appropriate (0-2, 3-8, 9-15, 15+) to represent critical periods of human growth and development.

5. The guidance provides a quantitative approach to account for the greater susceptibility of early-life exposure to chemicals that act through a mutagenic mode of action. An adjustment factor of 10 is applied to the cancer slope factor (derived from animal or epidemiology studies) for exposures before 2 years of age, a factor of 3 is applied for ages between 2 and 15 years, and no adjustment is applied after the age of 15. Please comment on whether the data and EPA analysis are scientifically sufficient to support these adjustment factors. Are sufficient data, including breadth of chemicals, available to make these determinations?

- The Panel suggests that the Agency consider alternative analyses, as discussed in the body of the report, that might allow them to use more of the available data and directly test hypotheses concerning the appropriateness of the adjustment values for predicting the dose-response from early exposure.

<u>Other Questions</u>

6. The Agency recognizes that consideration of children's risk is a rapidly developing area and, therefore, the Agency intends to issue future guidance that will further refine the present draft guidance and possibly address other modes of action as data become available. The Agency welcomes the SAB's recommendations on other modes of action that may be most fruitful to assess in similar future analyses.

- The Panel recommends that a priority for the near term would be the development of approaches for addressing early life exposures for agents whose mode of action is through endocrine disruption, beginning with estrogenic agents.

7. The analysis presented in the current Guidance relies on postnatal studies. Can the SAB recommend how to best incorporate data from transplacental or in utero exposure studies into future analyses?

- Due to the complexity of such an analysis, the Panel cannot recommend at this time a feasible method for incorporating transplacental/in utero exposure data. However, the Panel believes this to be an important issue that requires further research**.**

8. The Agency welcomes the SAB's recommendations on critical data needs that will facilitate the development of future guidance addressing differential lifestage susceptibility.

- The Panel recommends that the Agency work more closely with the research community to encourage the evaluation of early-life stage susceptibilities. For chemical agents known to increase cancer risk, carcinogenic potency and extent of exposure should be used in deciding which chemicals to study first.

- Additionally, the Panel suggests that the Agency reconsider limiting the application of adjustment factors only to mutagenic agents and instead apply a default approach to both mutagenic and to non-mutagenic chemicals for which mode of action remains unknown or insufficiently characterized.

INTRODUCTION

Background

In 1996, EPA published for public comment their proposed revisions to EPA's 1986 Guidelines for Carcinogen Risk Assessment (61 FR 17960, Apr. 23, 1996). Since the 1996 proposal, EPA's Science Advisory Board (SAB) has conducted three scientific peer reviews. In February 1997, the Science Advisory Board's (SAB) Environmental Health Committee (EHC) was asked to review the proposed revisions to the Agency's first cancer guidelines issued in 1986 (http://www.epa.gov/sab/pdf/ehc9710.pdf). In January 1999, the EHC met again to consider selected sections of the draft Guidelines that were revised to address recommendations from the public and the earlier SAB review (1997) of the Guideline. The revisions included new hazard descriptors and example narrative summaries; the expanded guidance on the use of Mode of Action information; the use of departure points for the dose-response analysis; and the approach to the Margin of Exposure analysis (http://www.epa.gov/sab/pdf/ec15.pdf). The EHC met for a third time in July 1999 to provide advice and comment to the EPA on issues related to applying the provisions of EPA's proposed revised Cancer Risk Assessment Guidelines (GLs) for children (http://www.epa.gov/sab/pdf/ec0016.pdf). The EHC has again been selected to lead the review

of the Supplemental Guidance due to its history in reviewing various documents associated with the EPA's Draft Cancer Guidelines and the relevance of the expertise of its members to this review.

According to the draft Supplemental Guidance, children's risk in the context of the Cancer Guidelines (U.S. EPA, 2003) includes early-life exposures that may result in both the occurrence of cancer during childhood and cancers that occur later in life. The relative rarity of childhood cancers and a lack of animal testing guidelines with perinatal exposure impede a full assessment of children's cancer risks from exposure to chemicals in the environment. "Perinatal" was defined as the time around birth and may include both prenatal (prior to birth) and postnatal (after birth) periods. The focus of the draft Supplemental Guidance is on childhood exposures resulting in cancer later in life.

The Supplemental Guidance for Assessing Cancer Susceptibility from Early-life Exposure to Carcinogens currently under review recognizes that the standard methodology to calculate cancer risk utilizes the lifetime average daily dose and accounts for differences between adults and children with respect to exposure factors, such as eating habits and body weight. However, susceptibility differences with respect to early lifestages are not currently taken into consideration because the cancer slope factors are based on effects observed following adult exposures. The purpose of this Supplemental Guidance is to provide a possible approach for assessing cancer susceptibility from early-life exposure to carcinogens. Since a much larger database exists for chemicals inducing cancer in adult humans or animals following mainly adult exposures, an analysis was undertaken to determine if adjustment of adult-based cancer slope

factors would be appropriate when assessing cancer risks from exposures early in life.The analysis undertaken addresses this issue, focusing upon studies that define the potential duration and degree of increased susceptibility arising from childhood (or early postnatal and juvenile animal) exposures.

The analysis was conducted to ascertain whether there are quantitative scientific data that would inform risk assessment policy choices for adjusting cancer slope factors based upon adult human epidemiology or standard chronic adult rodent bioassays in the assessment of cancer risk from childhood exposures. Thus, the critical data required are either human epidemiological data on childhood exposures resulting in adult cancer or research studies with rodents involving early postnatal exposures.

The Agency's review of the literature identified 28 studies (or groups of studies from a single laboratory on a given chemical) that directly provided quantitative data on carcinogenesis following early postnatal exposures and adult exposures to chemicals and radiation in animals. The carcinogenesis studies utilized 16 chemicals. Studies included in this analysis were those that reported tumor response from experiments that included both early-life and adult exposures. In addition, studies were identified for five other chemicals that showed early life-stage sensitivity with early postnatal exposure that were not evaluated quantitatively due to confounding factors related to experimental design.

The major available human data are from radiation exposures, with very limited data available for humans exposed during childhood to chemicals. A supporting role was assigned to

the available human radiation data, where cancer incidences in adults who were children at the time of the atomic bomb (A-bomb) exposure were compared with cancer incidences in adults who were older at the time of exposure. Although there are recognized differences in mechanism between radiation and mutagenic chemicals, the data on A-bomb survivors provide information in humans on many different cancer sites with a single exposure involving all ages. In addition to the richness of the data, a number of national and international committees of experts have analyzed and modeled these data to develop risk estimates for various specific applications.

The Panel concurs with the overall approach adopted by the Agency of using default adjustment factors to account for increased susceptibility due to early-life exposure, and the panel agrees that the values chosen for the cancer slope adjustment factors in the Supplemental Guidance appear to be reasonable from consideration of the literature. The Panel, however, suggests that the Agency improve the statistical analysis of the data and provide a more extensive discussion of how the Agency arrived at the choice of the 10x and 3x adjustment factors. The Agency should also make clear that these default adjustment factors would be used only when no data are available to directly assess cancer susceptibility from early-life exposure to a particular carcinogen. The Agency should consider conducting additional research to address this issue directly as suggested by several public presenters**.** After considering all relevant materials, both written and oral, the Panel provides below its comments and recommendations and has addressed each charge question individually.

Question 1

Please comment on whether the Agency's analysis as applied to chemicals acting through a mutagenic mode of action is accurate, reliable, unbiased and reproducible. Likewise, please comment on whether the underlying scientific information used to develop the guidance is accurate, reliable, unbiased and reproducible. Are there any key studies that the Agency has overlooked in reaching this conclusion?

PANEL RESPONSE TO QUESTION 1

Overall, the specific information and data selected, presented, and analyzed on the mutagenic mode of action appear accurate and reliable, and the presentation on the mutagenic agents was clear and concise. The Tables were for the most part self-explanatory. While quantitation of the differences in potency across life stages is difficult, the steps taken by this draft Supplemental Guidance – namely 1) the default assumption that early-life represents periods of increased susceptibility to mutagenic carcinogens, and 2) the quantification of the potency slope adjustment - are reasonable given the available data. It should be pointed out that this statement is made with the knowledge that the procedure established in the draft Supplemental Guidance for weighting carcinogens for early-life exposure is a default procedure to be used in the absence of chemical-specific information relevant to risk assessment following early life exposure. As noted in the Agency's carcinogen risk assessment guidelines, when you have chemical-specific data on early-life susceptibility (or lack thereof), that information should be used in the risk assessment of the specific carcinogen.

The assumption that mutagenic carcinogens are likely to be more potent when exposure occurs early in life is supported by a number of additional lines of inquiry not explicitly noted in the Supplemental Guidance. Indeed, the neonatal mouse model, used for decades, is known to be useful for detecting carcinogens with a mutagenic mode of action (McClain et al., 2001; Flammang et al., 1997). Studies have also shown elevated DNA-adduct formation in tissues from young animals exposed to mutagenic carcinogens relative to older animals (e.g., for vinyl chloride) (Laib et al. 1989; Morinello et al., 2002, others).

There are a large number of studies looking at the impacts of early-life exposure to carcinogens. Many of these studies, as well as the basic theories of carcinogenesis, point to the potential for early-life stages to be especially susceptible to chemicals acting through a mutagenic mode of action. Factors that contribute to this phenomenon may include, but are not limited to, differences by age in 1) cell division rate, 2) DNA repair capability, 3) state of differentiation and presence of stem cells, and 4) metabolic activating and detoxifying capability of tissues. These important factors differ in a growing and differentiating organism from a mature one, and differ at different stages of development. As noted by Swenberg et al. (1992) Anderson et al. (2000), Ginsberg (2003) and others, a major factor in early-life sensitivity to carcinogens is believed to be rapid cell division in growing and differentiating organisms. Mutations caused by carcinogens may be propagated if DNA repair does not occur before the cell divides. The rapid tissue growth and concomitant cell division can result in clonal expansion of initiated cells followed by promotion/progression to tumor formation. It has been observed that actively transcribing DNA is more prone to adduct formation (Thomale et al., 1994). DNA

repair can be deficient in fetal and neonatal tissues for some repair enzymes relative to adult organisms. This appears to be the case for alkyl-guanine alkyltransferase in neuronal tissues and likely plays a major role in the production of nervous system tumors by alkylating agents when exposure occurs early in life but not later in life (Rice and Ward, 1982; Naito et al., 1981; others). McConnell (1992) notes that perinatal exposure in conjunction with adult exposure usually increases the incidence of neoplasms and reduces the latency to tumor formation. Interestingly, this has also been observed for some non-mutagenic carcinogens.

Other Supporting Studies

There are many studies evaluating carcinogenesis after preconceptional exposure, transplacental exposure, lactational exposure, and early postnatal exposure to mutagenic carcinogens (reviewed in Anderson et al, 2000) not cited in the draft Supplemental Guidance. Although most of these investigations did not expose adults and juveniles in the same study, the data generally indicate increased early-life sensitivity when compared to results of studies in which exposure starts at maturity. This is manifested as higher tumor yield, earlier latency, and in some cases different tumor sites. At a minimum, one can say that these studies provide supporting evidence for use of a potency slope adjustment factor for early-life exposure to mutagenic carcinogens. For some mutagenic chemicals the highest tumor yields may be from prenatal exposure, in others from early postnatal exposure and still others from adult exposure (Anderson et al., 2000). In general, though, the studies reviewed by McConnell (1992) and Anderson et al., (2000) indicate that early-life exposure to mutagenic agents appears to result in increased potency of the carcinogen in question (higher tumor yield) and lower latency to effect

relative to later-life exposures alone. It should be noted that many studies also reported higher tumor incidence from exposure to non-mutagenic carcinogens when exposure starts early in life (DES, dieldrin, estragole, dioxin, others), and particularly when exposure continues through adulthood, which is the case for many environmental contaminants.

Many carcinogens require metabolic activation. The xenobiotic metabolizing enzymes of the liver and presumably other tissues have a generally lower level of activity and different isoforms prenatally as well as for some time postnatally (Cresteil et al, 1998; Milsap and Jusko, 1994; Snodgrass, 1992). Despite the apparently lower potential for metabolic activation in early-life, the susceptibility to carcinogenesis can be elevated in early life even when metabolic activation is required (e.g., benzo(a)pyrene).

Many investigations focused on prenatal exposure to carcinogens in order to shed light on mechanisms of carcinogenicity and the relationship between development and carcinogenesis. Relatively fewer studies evaluated early-life postnatal exposures and adult exposures in the same study or series of studies. Increased susceptibility in post-natal early-life to mutagenic carcinogens relative to adult exposures conducted in the same animal studies has been demonstrated for a number of compounds and agents including N-ethyl-N-nitrosourea (ENU), some polycyclic aromatic hydrocarbons, vinyl chloride, urethane, some nitrosamines, azoxymethane, amitrole, benzidine, and various types of radiation (see review by Anderson et al., 2000). Most of the key studies are cited in the draft Supplemental Guidance. Additional studies not cited in the Supplemental Guidance, which may describe relevant data useful for quantifying the adjustment factor, are provided at the end of the comments.

Radiation exposures to humans also provide data that indicate exposure to ionizing radiation early in life results in higher incidences of cancer relative to adult exposure for some tissues (thyroid, bone marrow, stomach, colon, lung, breast) (see Japanese survivor studies cited in EPA draft Supplemental Guidance; Miller, 1995), with evidence of specific windows of susceptibility (e.g., puberty for breast cancer risk from radiation treatment for Hodgkins lymphoma, Bhatia et al., 1996).

In addition, there are several studies not cited that have utilized infant mice in an initiation - promotion protocol (see references in appendix 2). These studies have demonstrated distinct gender, age, strain, and compound-related differences in the liver tumor promoting response in infant mouse. These data suggest a different mechanism of action for liver neoplasms in the infant treated mouse compared to the adult treated mouse. The Agency should expand the discussion of these data in this draft Supplemental Guidance as they illustrate a potential difference in the biology of the lesions induced in the neonatal mouse versus those induced in the adult mouse. If the lesions are different in their biology then that may infer a different mode of action. If this were the case, some guidance from the Agency on their prescribed course of action would be useful.

<u>Need for Better Explanation of Inclusion/Exclusion Criteria:</u>

As emphasized by some of the public commenters, the criteria for inclusion/exclusion of specific data in the analyses need clarification. The contexts in which data are collected to address a specific question define the bounds one must put on the interpretation of the results of

the analysis using the data. In very broad terms, data can fall into four specific areas: anecdotal, selective, comprehensive and representative. Representative data is the ultimate scientific goal in that an analysis of representative data, when done properly, should provide information on the distribution of possible outcomes in the general population of outcomes that can conceivably occur (you could estimate median, mean, percentiles, etc. and they would have meaning). Comprehensive data would encompass the collection of all possible data relating to an issue, which match some clearly defined criteria for what constitutes acceptable data. Comprehensive data are more difficult to interpret than representative data, but still provide distributional information that would be of value. Selective data refer to situations in which you select certain pieces of information because you feel they would give you some information on the range of possible outcomes that might occur. As such, selective data can be informative to the range of outcomes but are unlikely to inform the probability of a certain outcome occurring in the entire range of possible outcomes. Finally, anecdotal evidence can inform about the possibilities of a certain outcome, but gives only a very crude estimate for the possible range of outcomes. The toxicological data used by the EPA in the analysis of the factor to use in altering the slope for perinatal/childhood exposure is somewhere between anecdotal and selective and one must consider this in interpreting the findings from the evaluation.

As described in Section 2.1 of the Supplemental Guidance, the Agency chose to utilize studies in which exposures occurred during various life-stages in the same study. The reason for this is that such studies exclude problems with inter-study comparison, a valid concern. While that is a sound reason for including the studies that were analyzed, more effort should have been made to evaluate some of the excluded studies. There are studies not used in the EPA analysis in

which exposures of juvenile and mature animals to carcinogens occurred in the same study (see list at end of comments). The reason for exclusion of these studies is not always apparent.

The decision to select studies that compared tumor incidence between early life and adult exposures (page 11, paragraph 1) made for a more consistent database for the mutagenic, complete carcinogens examined. Other studies that have used neonatal and newborn exposure and measured neoplasm formation have not been utilized by design. The current restricted / selected references make for a less complete data set to examine the hypothesis that the young are more sensitive than adults to carcinogens than if all infant treatment papers were included. The database on which the mutagenic mode of action analysis was based came from predominantly one research group working with a mouse model. This might lead some to presume that the conclusions derived from the analysis are not generalizable; including additional studies would address this issue.

The Agency criteria used to select studies did not allow data available for mutagenic carcinogens where exposure occurred at different life-stages in the same species in multiple investigations to be used as indicated by public presenters. Extending the presentation of some of these data would help the argument that mutagenic carcinogens are likely to be more potent when exposure occurs early in life. If tumor incidence data following exposures at different life stages are available from different studies in the same strain, it would be reasonable and possible to use those data in the adjustment factor analysis.

Interstudy comparisons – Dosing regimen:

It appears that some studies were excluded from the analysis because the dose regimens at the early-life and mature stages were different. For instance, the data for tamoxifen-induced tumors in Wistar rats (Carthew et al., 1996, 2000), which demonstrated higher potency when given to juvenile rats relative to adult rats, were not used because of dose differences in the immature versus mature rats. It seems that an approach could be taken to evaluate these data as part of the analysis on appropriate adjustment factors. (See response to Question 5.)

The draft Supplemental Guidance (Page 15 and elsewhere) states that weekly food consumption rates and body weights generally were not available to allow more precise expression of the doses in terms of mg/kg for studies in which the carcinogen was dosed via the feed or drinking water. One could assume that the exposure itself did not affect food consumption or weight gain and use standard available data on typical values for the species in question. This might allow use of more of the available data for the analysis of the potency slope adjustment factors.

Different tumors at different age-of-exposure:

The last paragraph on page 22 indicates that early-life is a time of increased susceptibility to urethane induced lung adenomas, and that these tumors do not occur following exposure of adult animals. However, urethane induces other tumor types in adults. The potency of a carcinogen as utilized in risk assessment rarely comes with the caveat of being applicable only to

specific tumor sites. Many times there is little site concordance between species and sometimes this is the case within species by life stage. Standard risk assessment practice is to use the most sensitive site and sex as the basis of the potency factor. (There is also an argument for adding all sites together to assess cancer risk. This has been done for example in evaluating potency of asbestos to induce human tumors adding both lung cancer and mesothelioma risks together.) The Agency could consider evaluating the ratios of the dose that produced an early-life specific tumor type to the ratio for a later-life but different tumor type. This would be particularly appropriate if the most sensitive site in the early-life exposure in terms of potency is the site which does not develop tumors when exposure starts at maturity.

QUESTION 2.

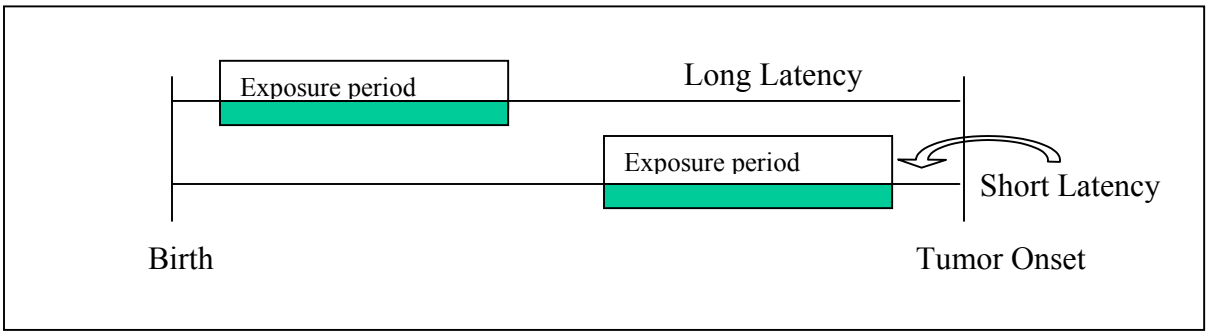
For chemicals acting through non-mutagenic modes of action, the Agency concludes that a range of approaches needs to be developed over time for addressing cancer risks from childhood exposures. Please comment on the Agency's conclusion that the scientific knowledge and data are insufficient at this time to develop generic guidance on how to address these chemicals and that a case-by-case approach is more suitable. Is the SAB aware of any additional data for chemicals acting through non-mutagenic modes of action relevant to possible early lifestage sensitivity?

PANEL RESPONSE TO QUESTION 2

The Panel agrees with the conclusion of the Agency that approaches need to be developed for agents with a known mode of action that is non-mutagenic (Tier 2b, Figure 3). The Panel disagrees with the Agency's conclusion that approaches and data are insufficient at this time to develop guidance on how to address non-mutagenic chemicals with an unknown mode of action (Tier 3, Figure 3). The Panel believes the data set for the non-mutagenic carcinogens to be qualitatively similar to that for the mutagenic carcinogens, although there are obvious deficiencies in both data sets, including small numbers of tumors overall and non-significant differences between adult and juvenile tumor incidences for some of the chemicals presented in the non-mutagenic data set. Although the non-mutagenic carcinogens differ widely in mechanism of action, the patterns of effects and the magnitudes of the ratios of juvenile versus adult incidences in the non-mutagenic data set do not differ appreciably from those in the data set for chemicals with a mutagenic mode of action. Therefore, the Panel believes that the Agency should consider the development and application of default adjustment factors for chemicals that are carcinogenic through an unknown mode of action (Tier 3, Figure 3).

Support for the proposition that early life exposure to carcinogens, regardless of the mode of action, results in increased incidence of tumors comes from the application of the time-dependent version of all multistage models of carcinogenesis. Assuming life expectancy is not dramatically affected, exposure for a fixed period early in life to a carcinogenic agent, compared to the same exposure later in life, provides a longer time window for any early stage effects to present themselves as detectable tumors (see Figure 1 below). This difference in latency is not currently incorporated into the Agency's guidelines. The slope adjustment factors chosen by the Agency will help to address these limitations in current risk assessment.

FIGURE 1



The Panel notes that for certain groups of chemicals that act by non-mutagenic modes of action, there is enough evidence supporting increased susceptibility to cancer with early life exposure that the Agency should include a discussion of these agents in the draft Supplemental Guidance. Although these chemicals may not be amenable to the quantitative analysis performed by the Agency, they serve as important examples in support of applying a default factor to non-mutagenic mode of action carcinogens when the mechanism of action is unknown.

According to the draft Supplemental Guidance (p18, paragraph 1), chemicals that are estrogen receptor agonists or antagonists, such as DES and tamoxifen, were not subjected to quantitative analysis by the Agency because no studies were available in which both juvenile and adult dosing occurred. However, multiple studies have been performed with both of these compounds, which observed increased reproductive tract tumors in rodents treated prenatally or during the neonatal period compared to an absence of such tumors with treatment during adulthood. For example, uterine, vaginal, and cervical cancers were observed with prenatal and neonatal exposure of mice to DES (McLachlan et al, 1980; Newbold et al, 1982; Newbold et al,

1990), whereas no such tumors were observed with lifetime exposure of adult mice (Highman et al, 1978). Although these observations come from different studies using different strains of mice, a review paper by Newbold (1995) cites unpublished data from her laboratory showing that acute treatment of adult mice does not result in uterine adenocarcinoma, whereas a similar treatment regimen during the neonatal period does cause adenocarcinoma. Presumably these studies would have been done in the same strain of mouse. The human data for DES support the animal data in that women who took DES did not develop vaginal adenocarcinoma or other cancers, but their daughters who were exposed *in utero* did develop vaginal adenocarcinoma. Other estrogen receptor agonists, including 17beta-estradiol (Newbold et al, 1990) and genistein (Newbold et al, 2001), have also been shown to induce uterine adenocarcinoma with treatment during the neonatal period. Perhaps with some minimal effort, the Agency may be able to obtain these expanded data as they move forward with known non-mutagenic modes of action.

Tamoxifen, an estrogen receptor agonist/antagonist, causes uterine adenocarcinoma when administered gestationally (Diwan et al, 1997) and neonatally (Carthew et al, 1997; Newbold et al, 1997) in rats and mice, whereas adult treatment (Carthew et al, 1996) does not. The 1996 and 2000 Carthew et al studies are cited in the draft Supplemental Guidance (p 18, para 1) as being inappropriate for quantitative analysis because of the very different doses used for adult and neonatal treatment (42mg/kg/d in adult rats versus 1mg/kg/d in neonatal rats). This seems to be missing the obvious point that uterine cancers were induced by dosing with a much lower dose for a much shorter interval in neonatal animals. However, the Carthew et al paper from 1996, states that the dose was actually 420 mg/kg of feed, whereas the Carthew et al 2000 study used gavage dosing. If the Agency estimated the daily dose based on average feed intake this should

be stated in the draft Supplemental Guidance (this would imply a food intake of 100 g/day, which seems high). Another statement on page 18, paragraph 1 of the draft Supplemental Guidance states that "the adult dosing period of only three months in the tamoxifen study potentially results in an overestimate of the early susceptibility compared with other adult studies with chronic dosing." This would seem to be incorrect for two reasons. First, the calculation of incidence per unit time of dosing presumably adjusts for this. Second, there were two adult dosing regimens used in this study, daily dosing for 3 months in rats or daily dosing from 8 weeks until 24 months in mice (Carthew et al, 1996). The authors report 4/24 animals with uterine tumors (two deciduomas, one hemangioma and one leiomyoma, but no adenocarcinomas) at 20 months age with the 3-month dosing regimen in rats and no tumors with the 24-month regimen in mice. The Panel offers the studies cited above as additional support for the assertion that there may be greater susceptibility to cancer development from early life-stage exposure to chemicals that act as estrogen receptor agonists than from adult exposure.

Dioxins and related compounds comprise another class of compounds about which more could be said in the draft Supplemental Guidance. Dioxins are known human carcinogens (IARC, 1997; USEPA, 2001). A recent publication on the Seveso cohort of humans exposed to dioxin as a result of an industrial explosion showed a significantly increased risk for breast cancer with increasing serum dioxin concentration obtained soon after the time of the explosion in 1976 (Warner et al, 2002). Animal bioassays have not shown increased mammary cancer with adult dioxin treatment (reviewed in USEPA, 2001), but a recent study by Brown et al (1998) found that gestational day 15 treatment with 1 μg/kg TCDD resulted in enhanced susceptibility to DMBA-induced mammary tumors. Similarly, neonatal treatment with 2.5 μg/kg TCDD on

postnatal day 18 was shown to enhance susceptibility to methylnitrosourea-induced mammary tumors (Desaulniers et al, 2001). Unfortunately, neither study evaluated a group treated only with TCDD perinatally for development of mammary tumors. Nonetheless, the data suggest that perinatal exposure to TCDD may increase susceptibility to the development of mammary cancers when compared with treatment only during adulthood**.**

In summary, the Panel agrees that the need for adjustment for early life-stage susceptibility for carcinogens acting through a known, non-mutagenic mode of action (Tier 2b in Supplemental Guidance Figure 3) should be evaluated by the Agency on a case-by-case basis. The Panel recommends that among this group of carcinogens, the Agency should consider developing guidance for carcinogens acting via estrogen receptor binding or other mechanisms that impact hormonally responsive tissues early in life. Particular consideration should be given to agents that may produce a persistent increase in susceptibility to cancer across multiple life stages following early life exposure. Finally, when the agent is non-mutagenic and the mode of action is unknown (Tier 3 in Supplemental Guidance Figure 2), the Agency has decided to implement a linear approach identical to that used for mutagenic agents. Because the data for non-mutagenic agents are qualitatively similar to the data seen for mutagenic agents and because the modeling approaches are identical, the Panel suggests that the agency reconsider the decision not to apply a default adjustment factor for the unknown mode, non-mutagenic agents.

QUESTION 3

Assuming that it is appropriate to conclude that there is a differential life stage susceptibility to chemicals acting through a mutagenic mode of action, the Agency's guidance uses a default approach that adjusts cancer slope factors (typically from conventional animal bioassays and/or epidemiological studies of adult exposure) to address the impact of early life stage exposure. Please comment on whether the approach is justified by the available data? Can the SAB suggest other approaches that might be equal or more appropriate?

PANEL RESPONSE TO QUESTION 3

The available studies analyzed adequately support a determination of increased early-life susceptibility to carcinogens. Despite the large number of carcinogens and considerable testing, the data available to allow quantification of any differential risk either broadly or for specific tumors in humans is limited. Increased risk will likely depend upon the cancer type. Simple multistage cancer models also predict that early-life exposures to early-stage carcinogens should increase total lifetime risk relative to later-life exposures. For later stage carcinogens the models suggest the opposite.

Because many carcinogens lack a comprehensive early-life data set the need exists for a default approach that in the absence of agent specific information adjusts for potentially increased early-life susceptibility. The data are strongest for mutagenic carcinogens largely because that database is more extensive, but are hard to distinguish from the general pattern seen for the non-mutagenic agents included in the analysis. The data set analyzed was restricted to chemicals for which multiple exposures in different life stages were available. However there is

a wealth of other individual chemical studies that support the basic premise of early life differences but do not allow a quantification of the differences. Thus, there is broader scientific support for differential susceptibility than reflected in the supplemental guidance. In recognition of this differential susceptibility, application of an adjustment to the adult cancer slope factor seems to be the most transparent and practical approach for risk assessment.

QUESTION 4

When considering differential susceptibility, the Agency's guidance separates the potential susceptible period into two age groups, 0 - 2 years and 2 - 15 years. These groupings were based on biological considerations rather than exposure considerations. The first grouping, 0 - 2 years of age, is meant to encompass a period of rapid development and the second grouping, 2 - 15 years of age, was selected to extend through middle adolescence approximately following the period of rapid developmental changes during puberty. Please comment on the scientific rationale that was used to justify these age groupings. Can the SAB suggest other plausible ways to make these groupings?

PANEL RESPONSE TO QUESTION 4

The Agency is proposing to adjust the risk estimates for adult cancer risks from early life exposures by incorporating two age groupings intended to capture increased periods of susceptibility: 0-2 years of age, and 2-15 years. The first group encompasses the period of most rapid growth and development (Gokhale and Kirschner 2003; Okasha et al., 2002). The second

group was selected to "represent middle adolescence appropriately following the period of rapid developmental changes during puberty". These recommendations were based on experimental data that compared the early life only *vs.* adult only and lifelong *vs.* adult only exposure periods.

The Panel believes that the guidelines would be strengthened by including more precise definitions of selected terms. The age categories need to be defined so that they are mutually exclusive. In addition, "adult" cancer risk is not well defined, other than to say that the focus of this draft Supplemental Guidance is on "…childhood exposures resulting in cancer later in life." (page 6).

Although there are significant physiological differences between pre-pubertal and pubertal children, there are limited data to indicate that the risk for development of cancers may be different in the two groups. Individuals during puberty may be more susceptible to some carcinogens than individuals at other life stages; consequently, the Panel concludes that a separate adjustment factor for the 9-15 year old group.

There has been a great deal of interest in the identification of critical windows of exposure as related to health outcomes in both children and adults. Several recent publications describe investigations of growth and development characteristics in childhood ("childhood exposures") and adult health outcomes, including cancer. Many of the studies assessing the impact of growth on subsequent health status have categorized growth into three phases, based on a model proposed by Karlberg et al. (Karlberg et al.,1987). Although the cut points used to define these three groupings vary somewhat across studies, generally the categories are defined

as 1) Infancy – from midgestation to age 2-3 years; 2) Childhood – from 3 years until "puberty"; and 3) Puberty (Gokhale and Kirschner 2003; Okasha et al., 2002; Hilakivi-Clarke et al., 2001; De Stavola et al., 2000). Moreover, the importance of growth velocity with respect to risk of subsequent adverse health outcomes, rather than absolute height and weight attained, is stressed in these investigations (Gokhale and Kirschner 2003;Okasha et al., 2002; De Stavola et al., 2000; Lofqvist et al., 2001). The relevance of these growth-related changes during each interval are described below. In order to better understand the implications of the rodent data, it would be helpful for the Agency to include a discussion of the relationships between developmental events in rodent species and humans. This would also allow for a closer comparison of the exposure and dose and effect data from rodent to human when available.

The birth to less than two years of age category

Growth occurs more rapidly during infancy than at any other interval over an individual's lifetime. Physiologic characteristics of importance relative to assessing risk for adult cancers are pronounced in infancy. During this period, there is a marked increase in linear growth and in the growth of all organs. For example, there is a significant increase in neuronal proliferation and maturation. The developing immune system may have a great impact on the ability to withstand environmental insults during this period (Klinnert et al., 2001).

The 2-8 years of age category

The 2-8 year old group represents a pre-pubertal period during which children grow at a linear rate of 5-6 cm per year (Grumbach, 2002). The rate of growth during the childhood phase is steady, although girls tend to grow in height and weight at a quicker pace than do boys and

achieve puberty earlier than their male counterparts. Hormonal influences on growth and development are of special interest in attempting to identify appropriate age groupings for risk assessment. Growth hormone stimulates both somatic and skeletal growth, particularly growth of the leg bones (Karlberg et al.,1987). Insulin–like growth factors (IGF-I) and thyroid hormones have also been shown to influence growth during this period (Robson et al, 2002; Lofqvist et al., 2001).

The 9-15 year old age group

The 9-15 year old age group represents the period of pubertal development during which dramatic increases in hormone levels result in growth and maturation of reproductive and other organs. The rate of linear growth and organ growth is much greater during this period than in the 2 to 8 year age group. It is acknowledged that there is variability both within and between genders with regard to the onset of puberty, emphasizing the differences in hormonal functioning according to age and gender. Other factors known to influence the age at onset of puberty include race/ethnicity and body mass index (Anderson et al., 2003; Karlberg, 2002; Rosenfield).

In males, there is very little secretion of gonadotropins by the pituitary gland until the age of 10 years, when secretion begins to increase steadily with the onset of puberty occurring at approximately 8-10 years of age (Grumbach, 2002). In females, the pituitary begins secreting progressively larger amounts of gonadotropic hormones at approximately eight years of age, with menarche occurring between ages 11 and 15 years, approximately two years after the onset of puberty.

Peak height velocity coincides with the onset of puberty in girls (around eight years of age) and in boys (around ten years of age) (Gokhale and Kirschner 2003;Grumbach and Styne 2002). Linear growth in young females continues but at a slower pace following menarche, with puberty ending when the breasts have reached the adult maturation stage; there is little continued gain in height after this period. In young males, puberty continues until age 18-20 years. Growth and development for both sexes is regulated by growth hormone and sex hormones; the marked increase in sex steroid secretion early in adolescence results in significant physiologic changes, including induction of serum binding proteins and detoxification enzymes (Grumbach, 2002).

The observation that puberty is a window of susceptibility for mammary tissue has been noted for ionizing radiation in the Japanese survivors and also in treatment for Hodgkins with radiation and chemotherapy during puberty (Bhatia et al., 1996) and possibly for tobacco smoke (Lash and Aschengrau, 1999; Morabia, et al., 2000). The draft Supplemental Guidance itself describes this phenomenon on page 23 for mammary tumors induced by DMBA in rats (Meranze et al., 1969; Russo et al., 1979). Increasing the slope adjustment factor for 9-15 year olds for reproductive organ and mammary gland carcinogens follows the logic in identifying early-life as a period of increased susceptibility due to rapid cell proliferation and the associated increased potential for clonal expansion of initiated cells.

In summary, we recommend that the 2-15 year age group be divided into pre-pubertal (age 2-8 years) and pubertal period (age 9-15 years). Since the risk for some tumors increases

with exposure to carcinogens during puberty, the agency should consider increasing the adjustment factor during this period.

QUESTION 5

The guidance provides a quantitative approach to account for the greater susceptibility of early-life exposure to chemicals that act through a mutagenic mode of action. An adjustment factor of 10 is applied to the cancer slope factor (derived from animal or epidemiology studies) for exposures before 2 years of age, a factor of 3 is applied for ages between 2 and 15 years, and no adjustment is applied after the age of 15. Please comment on whether the data and EPA analysis are scientifically sufficient to support these adjustment factors. Are sufficient data, including breadth of chemicals, available to make these determinations?

PANEL RESPONSE TO QUESTION 5

The values chosen for the cancer slope adjustment factors in the Supplemental Guidance appear to be reasonable from consideration of the literature. The Panel also suggests that the Agency improve the statistical analysis of the data (as discussed below) and provide a more extensive discussion of how they arrived at the choice of the 10X and 3X adjustment factors.

<u>The Data Used in Support of the Default Adjustment Factors</u>

Considering first the 10-fold slope adjustment factor for age 0-2 exposures, the data summarized in Table 4 and in Figures 1 and 2 (n=11 studies for chronic exposures) show that the median slope ratio for the *linear prevalence vs. dose model* is 10.0 with a range in ratios across the 11 individual studies of 0.3 to 65.0. Whether the median value of the distribution of 11 independent study results is an appropriate adjustment factor for modeling 0-2 age-specific exposure risks for mutagenic compounds is not clear. The public commenters have pointed to some unique features of the collection of studies that influence the derivation of this median value—many by a single investigator, common tumor sites (liver), the largest ratios are all obtained from studies that use male mice. By its nature as an estimate of central tendency in outcomes for the observed study data, it is a plausible value in the absence of actual age-specific dose-response data for a new compound.

The choice of a 3X multiplier for the slope adjustment factor for exposures during the age 2-15 year interval is derived entirely from a crude interpolation between the 1X factor for adults age 15+ and the 10X factor for infants age 0-2. Again this is a plausible factor given the study data that are available but other than conforming to intuitive, if not scientifically-substantiated bounds, there is no scientific basis in the analysis for choosing the factor of 3 over alternative values in this bounded range.

The draft Supplemental Guidance uses estimates of average excess relative risk (ERR) from atomic bomb survivor studies (Life Span Study) to support the premise of a life stage effect for mutagenic chemicals. These data strongly support this premise. For many types of cancer identified in the Life Span Study, estimates of ERR show an inverse relationship between

exposure and age at the time of exposure, i.e. younger people have a higher risk of cancer than older people. However, these estimates vary considerably with age among the various types of cancer. In some cases the 95% CI is large enough to include zero for all age categories (see mortality data in UNSCEAR Annex I). Thus, precise adjustment factors for younger age groups may be somewhat misleading without a discussion of uncertainties and limitations. Discussion should include the error associated with incidence data used to estimate ERR among the age groupings and the variation in ERR with age among the different types of cancer. For example, Table 9 in the report provides average ERR for four age groups. The trend clearly supports the premise that younger people have a higher risk of thyroid cancer, but the number of cases is small, and there is no indication of variance.

The ERR estimates cited in the draft Supplemental Guidance (Tables 8, 9, and 10) are based on cancer observed in populations exposed to large doses of radiation delivered at a high dose rate (UNSCEAR 2000). The original ERR estimates were based on a linear model applied through the entire dose range even though incidence data clearly are not linear over the entire dose range. Thompson et al. (1994) shows a large increase in incidence rate for all cancers in the >1 Sv cohort (mean dose of 1.6 Sv) but a small increase in incidence rate in the 0.01-0.99 Sv cohort (mean dose of 0.16 Sv). When broken down by cancer type, the number of cases per cohort per cancer type is very small, even zero in some cohorts. The draft Supplemental Guidance also ignores dose rate considerations. BEIR V provides a discussion of dose rate effectiveness factors for radiation. BEIR V appears in the reference list but does not appear to have been used in the text. Dose rate clearly affects risk. Consequently, the Supplemental

Guidance should include a discussion of the impact of dose and dose rate on the uncertainty associated with these risk estimates.

Thompson et al (1994) provide incidence rates among six age groupings for various types of cancer. However, the number of cases within many of the cohorts (including those for thyroid cancer) is very small; several of them have zero cases. This is particularly problematic for the high dose (>1 Sv) cohorts. Thompson et al (1994) estimated ERR at 1 Sv for each type of cancer by sex and age at exposure, but use of these estimates in the Supplemental Guidance needs to be accompanied by a discussion of the uncertainties. For example, the ERR for thyroid cancer in the 0-9 age group was 9.46 and for the 10-19 age group was 3.02. However, the 95% confidence interval for all ages was 0.48 - 2.14, once again pointing out the significance of uncertainty in the estimates.

Are the Analyses Used to Derive the Adjustment Factor Values Appropriate?

The analyses presented in the Supplemental Guidance are descriptive and use no formal statistical evaluations to test the selected adjustment values. Formal statistical procedures could have been used to more appropriately analyze individual study data; one such method is described in Halmes, Roberts, Tolson and Portier (2000). This analysis corrects for survival differences and differences in observation time, something not done in the EPA analysis and something which is likely to change the observed ratios. EPA is interested in whether the pattern of dose-response resulting from curve-fitting of the adult exposure data will, with their dosing correction and an appropriate factor change on the slope of the dose-response curve, predict the

dose-response seen from early life exposure. This is readily analyzed through direct statistical methods rather than a focus on only paired exposure groups. For example, EPA could apply their model choice to the combined perinatal/adult dose-response data and simply evaluate how often this hypothesis is rejected. However, given the limitations of the current data set, such an analysis is unlikely to substantially alter the general range of ratios seen in the supplementary guidance unless additional data could be used.

Note that in the Halmes et al paper, the majority of strictly early adult life exposures, when averaged over the lifespan of the animals, produced greater risk than predicted by the chronic exposure dose groups and no apparent difference existed between mutagenic and non-mutagenic exposures. While these analyses were done for data with early adult exposure rather than perinatal exposure, these findings support EPA's use of a slope adjustment in the perinatal period and suggest that non-mutagenic agents of unknown mode of action could also use a slope adjustment in early life.

Even assuming a full analysis as done by Halmes et al is not used here, the computation of the relative slope coefficients for juveniles and adults could have been done on the log-scale rather than the arithmetic scale. Since most models for cumulative incidence for tumor onset assume a functional form that includes an exponentiated dose function, changes in the point-of-departure for a fixed risk would better be reflected by a comparison of log-transformed data. The math is as follows:

$$P(dose)=1-[1-P(0)]\exp(-slope*dose) \quad [1]$$

Hence

{log[1-P(0)]-log[1-P(dose)]}/dose=slope [2]

This equation then implies that the ratio of the slopes would be the ratio of equation 2 for juveniles divided by equation 2 for adults. For small P(dose) and small P(0), the EPA formula is approximately equal to [2]; for medium range P(dose) as we have here, the equations are not the same. This transformation is nonlinear so the resulting ratios will be different.

If the EPA uses the analysis as presented in the Supplemental Guidelines, the exclusion of cases where the adults had no tumor and the juveniles had some tumors biases the median estimate of the resulting adjustment factors downward. Treating the division by zero as a big number, medians can still be calculated

The exclusion of cases where the adults had no tumor and the juveniles had some tumors (more than 10% of all tumors cited in the EPA data) potentially biases the median estimate of the resulting adjustment factors. This bias is likely to be in the direction of smaller ratios for medians, etc. The Agency is encouraged to expand their discussion and summarize the impact of these exclusions on the eventual choice for the slope adjustment. While the selected adjustment factors in the range of 10 and 3 for these age groups may be justified on the basis of weight of evidence, the data and analysis fall short of scientific justification.

QUESTION 6

The Agency recognizes that consideration of children's risk is a rapidly developing area and, therefore, the Agency intends to issue future guidance that will further refine the draft guidance and possibly address other modes of action as data become available. The Agency welcomes the SAB's recommendations on other modes of action that may be most fruitful to assess in similar future analyses.

PANEL RESPONSE TO QUESTION 6

Lifetime risk assessment appears to be little affected by changes in susceptibility that are limited in duration to the period of childhood itself, relative to the extant uncertainties and to the conservative assumptions made. This is not surprising in view of the relatively short duration of childhood vs. adult life. Effects in childhood that cause persistently elevated susceptibility throughout much or all of later life are likely to produce greater impacts on lifetime risk assessment and would be an appropriate focus for future research efforts. Further research needs to be undertaken to understand the circumstances under which early exposures to environmental agents may "re-program" (this term is intended to cover a diversity of mechanisms) cellular or organismal function(s) in a manner which increases future risk independent of ongoing exposure to the agent in question. While this mechanism may appear to be particularly relevant to hormonally active materials, it could result from other mechanisms such as the induction of long-term changes in cytochrome activity, alterations in cell population size, changes in cellular turnover rate, etc.

It is likely that early-life stages have windows of susceptibility to carcinogens acting through endocrine disruption. There are a number of studies that demonstrate susceptibility of early life stages to carcinogenesis by estrogen agonists/antagonists. Some of the studies on tamoxifen cited in the draft Supplemental Guidance are an example. Diethyl stilbestrol exposure *in-utero* produces female reproductive tract cancers in human offspring, without apparently increasing the risk of cancer in the mothers. Likewise, in animal models, both transplacental and *in utero* exposure to DES causes increased uterine adenocarcinomas and/or cervical cancers (Newbold et al., 1990). In addition, preconceptional exposure resulted in uterine cancers in the offspring (Newbold et al., 1998). In Newbold et al., 1990, the investigators tested other estrogenic compounds including hexestrol, trifluorodiethylstilbestrol and 17β –estradiol. The authors note that when the incidences of hyperplasia and adenocarcinoma were combined, the induction of these tumors and lesions followed the estrogenic potency of the compounds. The tumors were dependent on estrogen for growth in this study, as mice ovariectomized prior to puberty did not develop the tumors. Thus there is interplay between early-life exposure to estrogenic compounds and later pubertal development in terms of carcinogenesis.

Additional studies have evaluated the potential for carcinogenesis following perinatal exposure to tamoxifen, an estrogen antagonist in breast tissue but an estrogen agonist in uterine tissue. In addition to reproductive tract abnormalities, tamoxifen induced uterine adenocarcinomas and focal hyperplasias in mice following exposures the first five days after birth (Newbold et al., 1997). Induction of uterine tumors in adult mice was not observed in another study (Carthew et al., 1996). The soy phytoestrogen genistein is also capable of inducing

uterine adenocarcinoma in mice following postnatal exposure on days 1-5 (Newbold et al., 2001). Studies of tamoxifen effects following neonatal and adult exposures of Wistar rats indicate that the pups were more susceptible to uterine cancer induced by tamoxifen than the adult animals (Carthew et al., 1996; 2000). It should be noted that tamoxifen may be acting by multiple mechanisms as DNA-adducts in liver have been observed in rodent studies, and tamoxifen exposure to adult rats results in hepatocellular carcinoma. An additional example would be that of juvenile exposures to dioxin possibly increasing the potency of DMBA as a mammary tumorigen (discussed in the response to Question 2).

In summary, there is reason to believe that hormonal agents can be more potent carcinogens when exposure occurs in early-life stages than in later-life stages alone. This area is important to explore and the Agency should in future revisions of the Supplemental Guidance conduct an analysis of the differences in potency by age when data become available. As noted in the Guidance, three estrogen active agents are currently in test at NTP in multigenerational studies, and the results of those studies should shed light on early-lifestage susceptibility. We would also encourage the Agency to look at clinical data with secondary tumors arising from primary chemotherapy in children versus adults.

The proper addressing of additional modes of actions for young and infant animals will be dictated by the action of the particular chemical or physical carcinogen. Since this is still a developing area of research investigation in adult animals, the application to young and infant animals will require additional research investigations. These investigations, just like those

involving adult animals, should employ multiple doses to develop well-defined dose response characteristics for each chemical/physical agent.

The Agency might also look at the data on gene-environment interactions as they relate to polymorphisms in genes associated with xenobiotic metabolism and the critical windows of susceptibility. This may greatly enhance our understanding of these exposures and their relationship to cancer [in both childhood and adulthood] from a mechanistic point of view. A careful review of this literature linked to expression levels of the same enzymes compared between early life versus late life may be helpful in setting defaults for specific classes of agents.

CHARGE QUESTION 7

The analysis presented in the current Guidance relies on postnatal studies. Can the SAB recommend how to best incorporate data from transplacental or in utero exposure studies into future analyses?

PANEL RESPONSE TO QUESTION 7

No, the Panel cannot recommend a method to incorporate data from transplacental or in utero exposures at this time. However, we think this is an extremely important issue. It is clear from both human and animal studies that carcinogens can be transported across the placenta and induce tumor formation in the offspring. Clearly, use of DES as a therapeutic agent during

pregnancy resulted in vaginal cancers in daughters. Incorporating data from transplacental carcinogenesis studies is difficult but potentially important.

Studies that exposed animals prenatally and as adults have shown early-life sensitivity from *in utero* exposure to a number of mutagenic carcinogens including radiation (Delongchamp et al, 1997), benzene (Maltoni et al., 1989), vinyl chloride (Maltoni and Cotti, 1988), AZT (Olivera et al., 1997; Diwan et al., 1999), dibenzanthracene (Law, 1940), benzo(a)pyrene (Urso and Gengozian, 1982), arsenic (Waalkes, 2003), and a host of others (reviewed in Anderson et al, 2000).

DNA adducts have been measured in both animal embryos and human fetuses exposed to mutagenic carcinogens including polycyclic aromatic hydrocarbons (PAHs) (Arnould et al., 1997; Klopov, 1998; Autrup et al, 1995; Whyatt et al., 1998), vinyl chloride (Laib et al., 1989), ENU, and others. DNA adducts in the liver are higher after perinatal exposure to vinyl chloride than after exposure at maturity (Swenberg et al., 1992). In at least one study, PAH-DNA adduct levels were higher in white blood cells in the newborn human than the mother (Whyatt et al., 1998).

One possible approach to incorporating prenatal exposures in evaluating early-life sensitivity to carcinogenesis is to assess studies where both *in utero* and adult exposures were investigated in the same study. The review by Anderson, et al. (2000) that is cited in the Supplemental Guidance cites a number of papers that could be used in this type of analysis. Since the time of peak early-life sensitivity can be either pre- or postnatal, studies that evaluated

repeated prenatal, postnatal, and adult exposures would be the most useful for quantitative analysis of an adjustment factor for early-life exposure. Focusing on those studies might enable one to define the most sensitive period more clearly. However, quantifying the dose to the pups is difficult in these studies; that in turn makes quantitative evaluation of early-life susceptibility difficult. Thus, it seems unlikely that such studies will contribute data directly useful for quantitative risk assessment unless and until a marker or model of systemic exposure to the relevant material within the fetal compartment can be developed and validated. Application of physiologically-based pharmacokinetic (PBPK) modeling of transplacental transfer may prove fruitful although the models themselves are relatively undeveloped and require use of assumptions as much of the necessary data are unavailable. The Agency should, despite these difficulties, invest some effort in evaluating the prenatal studies as they may provide better evidence of peak developmental susceptibility. The evaluation could initially be qualitative and move over time towards a quantitative assessment as models are developed and new data are obtained.

The agency may wish to give early consideration to the manner in which such data are to be utilized. Specifically, such data could be used either on a chemical specific basis to establish individual chemical risks, or could be used to obtain a better understanding of the appropriate application of adjustments to exposure data obtained in later-life exposures. Because of differences in, for example, metabolic ontogeny between rodents and humans, it is not clear that early life exposure is, on a chemical by chemical basis, an appropriate model for quantitative human risk assessment. A more accurate and appropriate risk assessment may well be achieved by the application of biological understanding and quantitative adjustments obtained in

controlled, early-life experiments to later-life exposure data, very much like the current SGACS approach.

CHARGE QUESTION 8

The Agency welcomes the SAB's recommendations on critical data needs that will facilitate the development of future guidance addressing differential lifestage susceptibility.

PANEL RESPONSE TO QUESTION 8

There are rather large data gaps that need to be filled for the myriad of carcinogens that the Agency is charged with regulating. The majority of carcinogens have not been adequately tested in terms of early-life susceptibility. The Agency could work more closely with the research community to encourage the evaluation of early-life stage susceptibilities on a routine basis. Prioritization of carcinogens in the environment in terms of potency and extent of exposure would aid in deciding which chemicals to study first. The Agency should also partner with other federal agencies such as the CDC (to evaluate human exposures using monitoring data in order to inform the prioritization of chemicals for study) and FDA (which may have animal carcinogenicity studies on pharmaceuticals pertinent to the issue). Finally, the Agency could provide more resources to support the study of appropriate protocols for testing for early-life susceptibility to carcinogens with varying mechanisms of action.

Specific suggestions (not in priority order):

- The draft Supplemental Guidance relegates data on ionizing radiation to a supportive role. There is a large amount of published information, some of which EPA itself has generated, from human data on the Japanese bomb survivors that could possibly be used to improve the analysis. Since these analyses are of humans exposed to radiation, the uncertainty of inter-species extrapolation does not exist. Further, pharmacokinetic issues are moot for radiation exposures so these studies may provide a clearer view of the importance of pharmacodynamic factors. The data in Table 8 and 9 indicate that amongst the Japanese survivors of the atomic bomb the younger age groups were more sensitive than the adult age groupings to the induction of a number of cancers including thyroid, bone and connective tissue, skin, breast, and leukemia. The Agency should consider folding these data on ionizing radiation into the potency slope adjustment factor analysis and weighting them quantitatively.

- Additional research on adaptive responses in both adult and young is needed. Study of possible hormesis effects - protective effects at low dose – if known for the young should be explored. The state of the science in this field especially as it relates to infant / perinatal exposure should be incorporated in the draft Supplemental Guidance.

- There is a clear need to develop a better understanding of the biology and physiology of rodents typically used in carcinogenesis bioassays as they relate to similar phenomena in humans. The impacts of life-stage, gender, and related underlying physiological

differences in the animal models need to be related to similar changes in humans. Use of primate models, which more closely mimic lifestages in humans, may further the understanding of early life stage physiology and biology. In addressing life stage changes in physiology, key areas to address include the influence of hormonal levels and of phase I and phase II metabolic enzymes.

- Research is needed to better integrate our molecular understanding of carcinogenesis with life stages in humans and laboratory animal models. The use of genomics and proteomics in conjunction with bioinformatics holds promise for elucidating the many changes occurring in the cell/tissue/organ/organism during carcinogenesis as well as during development.

- There is a clear need for studies that address dosimetry issues. Studies using some of the compounds for which there appears to be evidence of increased early life stage sensitivity which are specifically designed to take into account the need for dose quantification and tumor latency could be performed, at least as relates to postnatal exposure. Such studies would probably require less-than-lifetime dosing during younger and older life stages, with multiple and similar times of sacrifice after onset of exposure to assess latency issues. As noted in the draft Supplemental Guidance, one would like to have studies with excellent quantitative data on tissue levels of test compound and its active metabolites in both exposed embryos/fetuses and exposed adults so that, following *in utero* exposures and adult exposures resulting in known target organ doses, the subsequent development

of cancers can be compared. Improved PBPK models would also be very useful in extrapolating internal doses.

- The Agency needs to look more towards models applicable to groups of chemicals related either structurally or by mechanism. Studies of prototypes of such groupings would be informative.

- Planning efforts currently underway by NICHD, EPA and NIEHS for the prospective National Children's Study (NCS) are directly relevant to the questions being posed here. If the NCS becomes a reality, there may be opportunity to examine physiological and biochemical changes that might relate to cancer susceptibility and improve the current Supplemental Guidance.

- In the future, the Agency should attempt to evaluate chemicals that are structurally similar to those chemicals that only produced tumors when exposure occurs early in life. These chemicals, while likely few in number, would be of great concern because the standard bioassay or typical occupational epidemiological study would not pick them up as carcinogens. Hence, such chemicals would not be treated as carcinogens by risk assessors. Perhaps the Agency can work towards identifying environmental chemicals that are structurally similar to the chemicals that only produce tumors when exposure occurs early in life for the risk assessor to consider. The Panel recommends that a more systematic effort be made to identify such chemicals and to define their characteristics.

APPENDIX 1

MISCELLANEOUS COMMENTS

Clarification of the Terms and Definitions used in the draft Supplemental Guidance

Many of the terms used in the draft Supplemental Guidance – for example mutagenesis, DNA reactive, genotoxic, nongenotoxic should be defined based on use in the draft Supplemental Guidance. This could be accomplished by including a glossary or appendix section with the definitions used. In addition, the term "mutagenic mode of action" should be more clearly defined, and consideration should be given to utilization of this term in the main guidance document to assure that either the usage is identical or that any differences in intended usage are made clear. It appears that the draft Supplemental Guidance considers a mutagenic mode of action if a chemical is carcinogenic and it is mutagenic in short-term bioassays. Several questions should be addressed; does DNA binding *in vivo* infer mutagenicity? Is mutagenic, DNA reactive, and genotoxic identical in use in this draft Supplemental Guidance? Each of these three terms has a specific identity associated with it and a specific mechanism and result. How will indirect mutagens, i.e. oxidative damage be considered? Along this line, with the DNA reactive carcinogens, mutation is not the only component of the mode of action involved in the neoplasm formation. Modulation of cell proliferation, apoptosis, and gene expression also participate in the development of the observed cancers and need to be considered and addressed in proposed modes of action for these chemicals.

Data for use in determining the mode of action

The Draft Supplemental Guidance should explicitly state the criteria for deciding that there are sufficient data to determine a particular agent's mode of action both in infant and adult animals (or at least refer back to the Cancer Risk Guidelines where these criteria are stated) Along these same lines, the Supplemental Guidance should comment on the quantity and quality of experimental evidence needed before a chemical is taken out of the default condition.

Tables

The tables do not indicate the reason for animal death in each study. Was the death due to chemically induced carcinomas or due to other organ failure? For example, Nitrosamines produce cirrhotic and general liver and kidney cytotoxicity in mice.

Was the tumor incidence expressed in the tables based on adenomas, carcinomas, combined adenomas and carcinomas? The tumor incidence values should specify the type of each tumor induced.

In several of the studies cited (Tables on pages 60, 62, 63, 64) no control groups were apparently utilized in the studies, making interpretation of the results difficult. This is a particular problem in trying to assess dose-response characteristics and threshold dose levels for the studies involved. Both parameters are needed in developing strong mode of action evaluations.

Tables 4 and 5

There are several errors. Staff should recheck data in tables against original papers and recalculate distribution of ratios; the errors found would probably not change the analysis significantly, although at least one ratio was off by a factor of 3 (in Table 4, DEN 6 ug/kg male mouse liver ratio should be 4.6, not 1.8).

Rounding should take place at the end, not the beginning; staff were inconsistent in doing this, sometimes rounding the percent incidences prior to calculating the ratios and sometimes not. One can get different calculated ratios, of course, when rounding at the beginning rather than the end.

Some of the citations are missing from the bibiliography, e.g., Vesselinovitch et al. 1983.

In the study by Meranze et al, 1969, exposures were evaluated in neonatal rodents, 5-8 week rodents and adults. The most sensitive period for mammary tumors occurred during the 5-8 week old period and undoubtedly represents development of the mammary gland during puberty in these animals. Ratios were calculated from data for both the neonatal compared to adult and for the adolescent compared to adults for total tumors and for mammary tumors in the female animals. It is not clear whether all those ratios were included in the analysis of the adjustment factors. In one Panelist's opinion, only the higher ratio for the female animals exposed at 5-8 weeks of age makes sense to include as that represents exposure during the more

sensitive postnatal time period for the females. To include the total tumor ratio as well actually dilutes the difference between adolescence and adult exposures for this tumor site.

The citation Maekawa et al, 1990 should really be Druckrey, 1970 as cited in Maekawa and Mitsumori, 1990. Also, the Maekawa and Mitsumori 1990 citation is missing from the bibliography.

The Agency should re-examine the way they utilized the data from Hard (1979). This study exposed rats to DMN at 3 weeks of age (earliest in this study), and at 4 weeks of age, as well as at 1.5, 2, and 3 months of age. The paper itself describes the 4-week old animals as juveniles (4 week old rats are still in adolescence), but the Agency treated them as adults in calculating the ratios used in the weighting analysis. The highest tumor incidences occurred in the 4 week old rats. If the ratio is recalculated treating these animals as juveniles, which is appropriate, then one gets slightly higher ratios when comparing the 6-week and older age groups.

A similar problem occurs when evaluating the data from Naito et al., 1981, although it is harder to "fix". In Naito et al., 1981, ENU was given to 1-day old, 1 week-, 2 week-, 3-week, and 4-week old rats. So, 4 weeks was the oldest animal group in this study, but the rats are still adolescents. Thus, the ratios calculated comparing the earlier age rodents with the 4 week old rodents may slightly underestimate the difference between immature and fully mature rodents in response to ENU with respect to neurological tumors. It is likely, though, that the underestimate would be slight because the induction of nervous tissue tumors by ENU appears to peak with

prenatal exposures and drop fairly rapidly postnatally (see Naito et al., 1981). This may have been recognized by the Agency and thus provides validity to the use of these data in the analysis of adjustment factors.

The proposed method of analysis does not take into account differences in multiplicity of tumors from early-life exposure. A number of studies have shown large differences in tumor multiplicity depending on the developmental stage of an organ in relation to timing of exposure (e.g., breast tumors in Meranze et al., 1969; lung tumors in a number of studies with urethane, nerve tissue tumors in a number of studies with ENU). Multiplicity of tumors in an organ is another indicator of susceptibility and would certainly be expected to influence disease outcome in both animals and humans. Thus, while it may be difficult to quantitatively weight multiplicity, it is certainly important to severity of disease, and an attempt should be made to weight multiplicity in future analyses.

Table 6

The reference by Vessilinovitch et al (1983) on amitrole is not included in the list of references.

The adult tumor incidence per time for ETU-induced thyroid tumors in female mice is incorrectly calculated as 0.02 due to an incorrect incidence rate in the control females being subtracted. The correct incidence/time is 4/96=0.04. This decreases the ratio from 10 to 5.

For PBB induced liver tumors in female mice, the adult dosing incidence for the 0:10 dosing regimen of 42/50 is used. For the male mice and female juvenile exposures the 30 ppm dose is used. The incidence from the 0:30 dosing regimen of 47/50 should be used instead, which would increase the adult incidence per time to 0.875 and reduce the ratio to 3.3.

APPENDIX 2

REFERENCES and/or DATA THAT EPA COULD EXPLORE FOR INCLUSION IN THE ANALYSIS:

Druckrey H and Lange A. 1972. Carcinogenicity of azoxymethane dependent on age in BD rats. Fed Proc 31(5):1482-4.

Druckrey H. 1973. Chemical structure and action in transplacental carcinogenesis and teratogenesis. IARC Sci Publ 4:45-58. (as reviewed in Anderson,2000; has data for azoxymethane induced nervous system tumors, dosed postnatal and adult rats, highest sensitivity is PN day 1)

Gray R, Peto R, Brantom P, and Grasso P. 1991. Chronic nitrosamine ingestion in 1040 rodents: the effect of the choice of nitrosamine, the species studied, and the age of starting exposure. Cancer Res (23 Pt 2):6470-91. (Is this same data as in Peto et al 84?)

Jurgelski W Jr, Hudson P, Falk HL. 1979. Tissue differentiation and susceptibility to embryonal tumor induction by ethylnitrosourea in the opossum. NCI Mongraph 51:123-158. (as reviewed in Anderson et al, 2000; found tumors with early postnatal days but not adults; opposum may be more equivalent to prenatal exposure)

O'Gara RW and Kelly,,MG. 1963 Comparative susceptibility of newborn, weanling, and adult mice to tumor induction by 3-methylcholanthrene and dibenz(ah)anthracene. Proceedings of the Association of Cancer Research 4:49.

Rice,JM and Ward, JM 1982. Age dependence of susceptibility to carinogenesis in the nervous system. Ann NY Acad Sci 381:274-89.

Rice IM. 1979. Problems and perspectives in perinatal carcinogenesis: a summary of the conference. NCI Monographs 51:271 (as cited in McConnell, 1992; discusses ENU given to pre-, postnatal and adult mice with subsequent kidney tumors, higher yield in animals exposed pre- and postnatally.)

Toth B. 1968. A critical review of experiments in chemical carcinogenesis using newborn animals. Cancer Res 28(4):727-38.

Vesselinovitch SD, Rao KV, and Mihailovitch N. 1975. Factors modulating benzidine carinogenicity bioassay. Cancer Res 35(10):2814-19. (dosed prenatal, postnatal, and adult mice with benzidine)

Vesselinovitch SD, Rao KV, Mihailovich N, Rice JM, and Lombard LS. 1974. Development of broad spectrum of tumors by ethylnitrosourea in mice and the modifying role of age, sex, and strain. Cancer Res 34(10):2530-38. (dosed multiple strains of mice prenatal, postnatal, and adults with ENU)

OTHER REFERENCES:

Infant mouse initiation promotion protocols

Klaunig JE. Pereira MA. Ruch RJ. Weghorst CM. Dose-response relationship of diethylnitrosamine-initiated tumors in neonatal balb/c mice: effect of phenobarbital promotion. Toxicologic Pathology. 16(3):381-5, 1988.

Lee GH. Ooasa T. Osanai M. Mechanism of the paradoxical, inhibitory effect of Phenobarbital on hepatocarcinogenesis initiated in infant B6C3F1 mice with diethylnitrosamine. Cancer Research. 58(8):1665-9,1998 Apr 15.

Siglin JC. Weghorst CM. Rodwell DE. Klaunig JEGender- dependent differences in hepatic tumor promotion in diethylnitrosamine initiated infant B6C3F1 mice by alpha-hexachlorocyclohexane. Journal of Toxicology & Environmental Health. 44(2):235-45, 1995 Feb.

Weghorst CM. Devor DE. Henneman JR. Ward JM. Promotion of hepatocellular foci and adenomas by di(2-ethylhexyl) phthalate and phenobarbital in C3H/HeNCr mice following exposure to N-nitrosodiethylamine at 15 days of age. Experimental & Toxicologic Pathology. 45(7):423-31, 1994

Weghorst CM. Pereira MA. Klaunig JE. Strain differences in hepatic tumor promotion by phenobarbital in diethylnitrosamine- and dimethylnitrosamine-initiated infant male mice. Carcinogenesis. 10(8):1409-12, 1989.

Weghorst CM. Pereira MA. Klaunig JE. Strain differences in hepatic tumor promotion by phenobarbital in diethylnitrosamine- and dimethylnitrosamine-initiated infant male mice. Carcinogenesis. 10(8):1409-12, 1989 Aug.

Weghorst CM. Klaunig JE. Phenobarbital promotion in diethylnitrosamine-initiated infant B6C3F1 mice: influence of gender. Carcinogenesis. 10(3):609-12, 1989

REFERENCES CITED IN COMMENTS:

Anderson SE, Dallal GE, Must A. 2003. Relative weight and race influence average age at menarche: results from two nationally representative surveys of US girls studied 25 years apart. Pediatrics.111(4 Pt 1):844-50.

Anderson LM, Diwan BA, Fear NT, and Roman E. 2000. Critical windows of exposure for children's health: Cancer in human epidemiological studies and neoplasms in experimental animal models. Environ Health Perspect 108(Suppl 3):573-594.

Arnould JP, Verhoest P, Bach V et al. 1997. Detection of benzo(a)pyrene-DNA adducts in human placenta and umbilical cord blood. Human and Experimental Toxicology 16(12):716-721.

Autrup H, Vestergaard AB, Okkels H. 1995. Transplacental transfer of environmental genotoxins:polycyclic aromatic hydrocarbon-albumin in nonsmoking women, and the effect of maternal GSTm1 genotype. Carcinogenesis 16(6):1305-9.

Bhatia S, Robison LL, Oberlin O, et al. 1996. Breast cancer and other second neoplasms after childhood Hodgkin's disease. NEJM 334:745-751.

Brown NM, Mansolillo PA, Zhang J-X, Wang J, Lamartiniere CA. 1998. Prenatal TCDD and predisposition to mammary cancer in the rat. Carcinogenesis 19:1623-1629.

Carthew P, Edwards RE, Nolan BM et al. 1996. Tamoxifen associated uterine pathology in rodents:relevance to women. Carcinogenesis17:1577-1582.

Carthew et al, 1997, Carcinogenesis 21: 797

Carthew P, Edwards RE, Nolan BM et al. 2000. Tamoxifen induces endometrial and vaginal cancer in rats in the absence of endometrial hyperplasia. Carinogenesis 21:793-797.

Cresteil T. 1998. Onset of xenobiotic metabolism in children: toxicological implications. Food Additiv Contamin 15 (suppl):45-51.

Delongchamp RD, Mabuchi K, Yoshimoto Y, and Preston DL. 1997. Cancer mortality among atomic bomb survivors exposed in utero or as young children, October 1950-May 1992. Radiat Res 147:385-395.

Desaulniers D, Leingartner K, Russo J, Perkins G, Chittim BG, Archer MC, Wade M, Yang J. 2001. Modulatory effects of neonatal exposure to TCDD, or a mixture of PCBs, p,p'-DDT, and p-p'-DDE, on methylnitrosourea-induced mammary tumor development in the rat. Environ Health Perspect 109:739-747.

De Stavola BL, Hardy R, Kuh D, dos Santos Silva I, Wadsworth M, Swerdlow AJ. 2000. Birthweight, childhood growth and risk of breast cancer. Br J Cancer 83:964-68.

Diwan BA, Anderson LM, Ward JM. 1997. Proliferative lesions of oviduct and uterus in CD-1 mice exposed prenatally to tamoxifen. Carcinogenesis 18:20092014

Diwan BA, Riggs CW, Logsdon D, Haines DC, Olivera OA, Rice JM, Yuspa SH, Poirier M, and Anderson LM. 1999. Multiorgan transplacental and neonatal carcinogenicity of 3'-azido-3'-deoxythymidine in mice. Toxicol Appl Pharmacol 161:82-99.

Drew RT, Boorman GA, Haseman JK, McConnell EE, Busey WM, and Moore JA. 1983. The effect of age and exposure duration on cancer induction by a known carcinogen in rats, mice, and hamsters. Toxicol Applied Pharmacol 68:120-130.

Flammang TJ, Von Tungeln LS, Kadlubar FF, and Fu PP. 1997. Neonatal mouse assay for tumorigenicity: Alternative to the chronic rodent bioassay. Reg Toxicol Pharmacol 26:230-240.

Ginsberg, G. 2003. Assessing cancer risk from short-term exposures in children. Risk Analysis 23:19-34.

Gokhale R and Kirschner BS. 2003. Assessment of growth and nutrition. Best Practice and Research Clinical Gastroenterology. 17:153-62.

Grumbach, MM, 2002. The neuroendocrinology of human pubert revisited Hormone Research 57, Suppl 2: 2-14.

Grumbach, MM and Styne, DM, 2002. Puberty: Ontogeny, Neuroendocrinology, Physiology and Disorders. In Williams Textbook of Endocrinology, P.R. Larsen, H.M. Kronenberg, S. Melmed and K. Polansky, editors Saunders, Philadelphia, pages 1115-1286.

Halmes NC, Roberts SM, Tolson JK, and Portier, CJ. 2000. Reevaluating cancer risk estimates for short-term exposure scenarios. Toxicological Sci 58:32-42.

Hard G. 1979. Effect of age at treatment on incidence and type of renal neoplasm induced in the rat by a single dose of dimethylnitrosamine. Cancer Research 39:4965-4970.

Hilakivi-Clarke L, Cho E, deAssis S, Olivo S, Ealley E, Bouker KB, Welch JN, Khan G, Clarke R, Cabanes A. 2001. Maternal and prepubertal diet, mammary development and breast cancer risk. Am Soc Nutr Sci J Nutr. 131:154S-57S.

Highman B, Norvell MJ, Shellenberger TE, 1978. Pathological changes in female C3H mice continuously fed diets containing diethylstilbestrol or 17B-estradiol. J Environ Pathol Toxicol 1:1-30

IARC 1997. Polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans. IARC Monogr Eval Carcinog Risk Hum 69:33-344

Karlberg J, Engstron I, Karlberg P, Fryer JG. 1987. Analyses of linear growth using a mathematical model. Acta Paediatrica Scandinavica 76(Supplement):478-88.

Karlberg J. 2002. Secular trends in pubertal development. Horm Res 57(suppl 2):19-30.

Klinnert MD, Nelson HS, Price MR, Adinoff AD, Leung DY, Mrazek DA. 2001. Onset and persistence of childhood asthma: predictors from infancy. Pediatrics 108:E69.

Klopov VP. 1998. Persistent organic compounds in women residing in the Russian arctic. International Journal of Circumpolar Health 57 Suppl 1:555-60.

Laib RJ, Bolt HM, Cartier R, and Bartsch H. 1989. Increased alkylation of liver DNA and cell turnover in young versus old rats exposed to vinyl chloride correlates with cancer susceptibility. Toxicol Lett 45:231-239.

Lash TL and Aschengrau A 1999. Active and passive cigarette smoking and the occurrence of breast cancer. Am J Epidemiol 149:5-12.

Law LW. 1940. The production of tumors by injection of a carcinogen into the amniotic fluid of mice. Science 91:96-97.

Liebert RA, Liebert AG, and Lane M. 1964. Hormonal influences on urethan carcinogenesis in C3H/f mice. Cancer Research 24:186901879.

Lofqvist C, Andersson E, Gelander L, Rosberg S, Blum WF, Wikland KA. 2001. Reference values for IGF-I throughout childhood and adolescence: A model that accounts simultaneously for the effect of gender, age, and puberty. Endocrinol Metab 86:5870-76.

Maekawa A and Mitsumori K. 1990. Spontaneous occurrence and chemical induction of neurogenic tumors in rats – influence of host factors and specificity of chemical structure. Clin Rev Toxicol. 20:287-310.

Maltoni C, Cotti G. 1988. Carcinogenicity of vinyl chloride in Sprague-Dawley rats after prenatal and postnatal exposure. Ann NY Acad Sci 534:145-159.

Maltoni C, Ciliberti A, Cotti G, Conti B, Belpoggi F. 1989. Benzene, an experimental multipotential carcinogen: results of the long-term bioassays performed at the Bologna Institute of Oncology. Environ Health Perspect 82:109-124.

Maltoni C, Lefemine G, Ciliberti A, Cotti G, and carretti D. 1981. Carcinogenicity bioassays of vinyl chloride monomer: A model of risk assessment on an experimental basis. Environ Health Perspect 41:3-29.

McClain RM, Keller D, Casciano D, Fu P, MacDonald J, Popp, J, and Sagartz J. 2001. Neonatal mouse model: Review of methods and results. Toxicologic Pathol 29(Suppl.):128-137.

McLachlan JA, Newbold RR, Bullock BC. 1980. Long-term effects on the female mouse genital tract associated with prenatal exposure to diethylstilbestrol. Cancer Res 40:3988-3999.

Meranze DR, Gruenstein M, and Shimkin MB. 1969. Effect of age and sex on the development of neoplasms in Wistar rats receiving a single intragastric instillation of 7,12-dimethylbenz(a)anthracene. Int J Cancer 4:480-486.

Miller RW. 1995. Special susceptibility of the child to certain radiation-induced cancers. Environ Health Perspect 103(Suppl 6):41-44.

Milsap RL and Jusko WJ. 1994. Pharmacokinetics in the infant. Environ Health Perspect 102(Suppl 11):107-110.

Morinello EJ, Ham A-JL, Ranasinghe A, Nakamura J, Upton PB, and Swenberg JA. 2002. Molecular dosimetry and repair of N2,3-ethenoguanine in rats exposed to vinyl chloride. Cancer Research 62:5189-5195.

Naito M, Naito Y, and Ito A. 1981. Effect of age at treatment on the incidence and location of neurogenic tumors induced in Wistar rats by a single dose of N-ethyl_N-nitrosourea. Gann 72:569-577.

Neal J and Rigdon RH. 1967. Gastric tumors in mice fed benzo(a)pyrene: A quantitative study. Texas Reports on Biology and Medicine 25:553-557.

Newbold RR, Padilla-Banks E, Bullock B, and Jefferson WN. 2001. Uterine adenocarcinoma in mice treated neonatally with genistein. Cancer Research 61:4325-4328.

Newbold RR, Jefferson WN, Padilla-Burgos E, Bullock BC. 1997. Uterine carcinoma in mice treated neonatally with Tamoxifen. Carcinogenesis 18(12):2293-2298.

Newbold RR, Bullock BC, McLachlan JA. 1990. Uterine adenocarcioma in mice following developmental treatment with estrogens: a model for hormonal carcinogenesis.

Newbold RR, Hanson RB, Jefferson WN et al. 1998. Increased tumors but uncompromised fertility in the female descendants of mice exposed developmentally to diethylstilbestrol. Carcinogenesis 19:1655-1663.

Newbold R. 1995 Cellular and molecular effects of developmental exposure to diethylstilbestrol: Implications for other environmental estrogens. Environ Health Perspect 103(Supp 7):83-87.

Newbold RR, McLachlan JA. 1982 Vaginal adenosis and adenocarcinoma in mice exposed prenatally or neonatally to diethylstilbestrol. Cancer Res 42:2003-2011.

Okasha M, Gunnell D, Holly J, Smith GD. 2002. Childhood growth and cancer. Best Practice and Research Clinical Gastroenterology. 16:225-41.

Olivera OA, Anderson LM, Diwan BA, Haines DC, Harbaugh SW, Moskal TJ, Jones AB, Rice JM, Riggs CW, Logsdon D, Yuspa SD, and Poirier MC. 1997. Transplacental effects of 3'-

azido-3'-deoxythymidine (AZT): Tumorigenicity in mice and genotoxicity in mice and monkeys. JNCI 89:1602-08.

Peto R, Gray R, Brantom P and Grasso P. 1984. Nitrosamine carcinogenesis in 5120 rodents: chronic administration of sixteen different concentrations on NDEA, NDMA, NPYR, and NPIP in the water of 4440 inbred rats, with parallel studies on NDEA alone of the effect of age of starting (3,6, or 20 weeks) and of species (rats, mice, or hamsters). IARC Sci Publ 57:627-665.

Pietra G, Rappaport H, Shubik P. 1961. The effects of carcinogenic chemicals in newborn mice. Cancer 14:308-317.

Rice,JM and Ward, JM 1982. Age dependence of susceptibility to carinogenesis in the nervous system. Ann NY Acad Sci 381:274-89.

Robson H, Siebler T, Shalet SM, Williams GR. 2002. Interactions between GH, IGF-I, glucocorticoids, and thyroid hormones during skeletal growth. Pediatr Res. 52:137-47.

Rogers S. 1950. Age of the host and other factors affecting the production with urethane of pulmonary adenoms in mice. J Experimental Medicine 93:427-49.

Rosenfield RL, Puberty in the Female and its Disorders. In Pediatric Endocrinology, M. Sperling, editor Saunders, Philadelphia, pages 455-518.

Russo J, Wilgus G, and Russo IH. 1979. Susceptibility of the mammary gland to carinogenesis. I. Differentiation of the mammary gland as a determinant of tumor incidence and type of lesion. Am J Pathol 96:721-736.

Swenberg JA, Fedtke N, Fishbein L 1992. Age-related differences in DNA adduct formation and carcinogenesis of vinyl chloride in rats. In Similarities and Differences Between Children and Adults: Implications for Risk Assessment. P.S. Guzelian, C.J. Henry, and S.S. Olin, Eds. ILSI Press, Washington D.C.

Thomale J, Hochleitner K, and Rajewski M.F. 1994. Differential formation and repair of the mutagenic DNA alkylation product O6-alkylguanine in transcribed and nontranscribed genes of the rat. J Biol Chem 269:1681-86.

Thompson, D.E. et al. 1994. Cancer Incidence in Atomic Bomb Survivors. Part II: Solid Tumors, 1958-1987. Radiat. Res. 137: S17-S67.

Urso P, and Gengozian N. 1982. Alterations in the humoral immune response and tumor frequencies in mice exposed to benzo(a)pyrene and X-rays before or after birth. J Toxicol Environ Health 10:817-835.

US EPA 2001 Dioxin Reassessment. US Environmental Protection Agency.
http/cfpub.epa.gov/ncea/cfm/diox

Vesselinovitch SD, Kyriazis AP, Mihailovich N, and Rao KVN. 1975. Conditions modifying development of tumors in mice at various sites by benzo(a)pyrene. Cancer Research 35:2948-53.

Vesselinovitch SD, Rao KVD, and Mihailovich N. 1979. Neoplastic response of mouse tissues during perinatal age periods and its significance in chemical carcinogenesis. NCI Monograph 51:239-249.

Vessilinovitch SD, Koka M, Mihailovitch N, and Rao KVN. 1984. Carcinogenicity of diethylnitrosamine in newborn, infant, and adult mice. J Cancer Res Clin Oncol 108:60-65.

Waalkes MP, Ward JM, Liu J, Diwan BA. 2003. Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. Toxicol Appl Pharmacol 186:7-17.

Walters MA. 1966. The induction of tumours by the injection of 9,10-dimethyl-1,2-benzanthracene (DMBA) into newborn sucking and young adult mice. A dose response study. Br J Cancer 20:148-160.

Warner M, Eskenazi B, Mocarelli P, Gerthoux PM, Samuels S, Needham L, Patterson D, Brambilla P 2002 Serum dioxin concentration and breast cancer risk in Seveso Women's Health Study. Environ Health Perspect 110:625-628

Whyatt RM, Bell DA, Jedrychowski W et al. 1998. Polycyclic aromatic hydrocarbon-DNA adducts in human placenta and modulation by CYP1a1 induction and genotype. Carcinogenesis 19(8):1389-92.